Courtship behaviour in the spider *Pityohyphantes phrygianus* (Linyphiidae, Araneae): do females discriminate injured males?

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Courtship behaviour in the spider *Pityohyphantes phrygianus* (Linyphiidae, Araneae): do females discriminate injured males? - Courtship behaviour was studied in the sheetweb spider *Pityohyphantes phrygianus* (C.L.Koch). The courtship, involving virgin females and previously unmated males, consists of three major phases with regard to male behaviour: (1) shaking of abdomen when touching the female's web; (2) reduction of the web area; and (3) pseudocopulation. The female is mainly inactive during courtship but raises her abdomen from a horizontal position before the male terminates web reduction.

In wild populations, males with leg injuries are common; approximately 16% of the males in the study population showed such injuries. Therefore, in experimental males, one of the forelegs was shortened 44% before courtship. Males with asymmetric legs performed the initial phase of pseudocopulation less well than normal males. Injured males had difficulties in finding a suitable position and the number of short breaks at the beginning of pseudocopulation were more frequent than in normal males. Consequently, experimental pairs spent a longer time finding a stable mating position. However, no female discrimination of males with an amputated leg was observed. It may be potentially dangerous for females to reject a male since the sex ratio, both primary and secondary, is highly female biased in natural populations.

Key-words: Female choice - courtship behaviour - injured males - asymmetry - spider - web reduction.

INTRODUCTION

The function of spider courtship has been debated ever since late 19th century (ROBINSON 1982). One hypothesis is that the courtship provides the female with information about male quality, i.e. intersexual selection (ROBINSON 1982, WATSON 1990).

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Manuscript accepted 09.01.1996.

Proceedings of the XIIIth International Congress of Arachnology, Geneva, 3-8.1X.1995.

Leg injuries among males are common in natural spider populations (unpubl. obs.). Such injuries may arise in several ways. Many specimens save themselves from a predator's attack by separating a leg from the rest of the body (FORMANOWICZ 1990). Furthermore, fighting between males may result in loss of forelegs (DODSON & BECK 1993). If a leg is broken at a joint, the hemolymph pressure forces the joint membrane to seal the wound (FOELIX 1982). Shortened legs in the adult stage may also be caused by incomplete, or partly unsuccessful moulting (Gunnarsson unpubl.). Thus, it is possible that females may use injured legs as a cue for assessing male quality. Alternatively, the females do not discriminate damaged males because other aspects of their mate are more important.

If injured males perform a courtship that is different from the behaviour of normal specimens, females may reject such males. A rejection is plausible if the behaviour signals inferior quality or if the female experiences higher risks, e.g. due to predation during courtship. However, another alternative is that females do not discriminate between injured and normal males. This would be expected if the altered male courtship behaviour does not affect female fitness in any way, or if the females are unable to detect the injury, or if females for some reason mate with the first available male. The latter expectation would e.g. be probable if the operational sex ratio is female biased.

In the present study, we examined the courtship behaviour in normal and injured males of the sheet web spider, *Pityohyphantes phrygianus* (C.L. Koch, 1870) and the females' response to courtship. Injured males were produced by shortening one foreleg by approximately 44%. We define courtship as the male behaviour from detecting the female's web until the onset of sperm transfer. Given that visual signals are of little importance in many spider families, including linyphids, the web seems to provide the male with some information about the reproductive status of the female (unpubl. obs.). The copulation in linyphids is preceded by a pseudocopulation, where normal copulation behaviour is performed but no sperm is transferred (VAN HELSDINGEN 1965, AUSTAD 1982, SUTER 1990). We included this behaviour in our study of the courtship in *P. phrygianus*.

METHODS

Subadult spiders were collected at two coniferous forest sites in SW Sweden about 20 and 30 km east of Göteborg. The collecting was done between late March and mid April 1993. We enclosed the needle-carrying parts of spruce branches in a plastic sack and shook the branches vigorously. The spiders were sorted out from the debris and were kept separately in plastic vials closed with a cotton ball.

In the laboratory the specimens were initially kept at 4°C. After 10-44 days they were brought to room temperature (20°C). Before moulting the males were randomly placed in three groups: (1) untreated males (N=19), (2) males that were anaesthetized with carbondioxide (N=8), and (3) males that were anaesthetized and had one foreleg surgically amputated with a scalpel just below the patella (N=20), i.e. tibia, metatarsus and tarsus were removed. All treatments were made within two days.

Whether the left or right leg was amputated was decided at random. The amputated legs were not regenerated after the final moulting. Five specimens in group (3) were adults when the leg was amputated. All specimens survived the amputation treatment and no decreased vigour was observed.

The time until moulting in room temperature was 3-10 days for females and 1-3 days for males (cf. GUNNARSSON & JOHNSSON 1990). After treatment and moulting the specimens were kept in 10°C until the experiment started. The courtship experiments were conducted on May 10-19 and 29, 1993. The females were brought to room temperature 72 h before the experiment started and each of them was placed in a 500 ml plastic vial equipped with spruce twigs. In each trial (N=47), one previously unmated male was introduced into the web of a virgin female. Each individual was used only once. When introduced, males were carefully placed in the web and as far away from the female as possible. The courtship was considered to have started when the male shook his abdomen for the first time. Shaking consisted of a rapid vertical jerking of the abdomen and this time was set as t=0. The spiders were observed until a pseudocopulation had lasted for 50 minutes without interruptions, or until three hours had elapsed. Special attention was given to the female's behaviour, i.e. the time the female raised her abdomen, and the start of pseudocopulation. After 24 h we separated the pairs and the specimens were weighed. Female weight was 7.54 \pm 1.25 mg (N=43) and male weight was 7.56 \pm 1.37 mg (N=42). There were no weight differences between groups (1)-(3), either among females or males (Kruskal-Wallis ANOVA, P=0.80, N=43 and P=0.59, N=42, respectively).

No food was provided before or during the experiment, but water was available in the vials. After termination of courtship experiments, 10 females from group (1), 4 females from group (2), and 11 females from group (3) were randomly choosen. These specimens were fed, and we recorded oviposition and hatching success.

We used non-parametric statistics in the analyses because we suspected nonnormal distribution of the data. All means are given together with their S.D., unless otherwise stated. Sample size in the text refers to total number of specimens in each group.

RESULTS

Courtship of normal males

All males except of one carried out courtship behaviour. When introduced into the female's web, the male's courtship started almost immediately. Within two minutes 89% of the males began to shake their abdomen; the mean time was 3.3 ± 9.5 min. (N=18). The shaking was of short duration and was followed by a period of quiescence (Fig. 1). The female remained inactive during this first phase.

The second phase of courtship consisted of a reduction of the web area (Fig. 1). Biting off all but a few of the supporting silk threads, the male reduced the sheet web to a small wad. During the web reduction phase, the male approached the female



Male Treatment



Timing of courtship activities. First phase: male shaking his abdomen (\blacksquare). Second phase: web reduction (\square) and female raising her abdomen (\bullet). Third phase: pseudocupulation, with short breaks (\blacksquare), and continued for at least 50 min. (\blacksquare). Phases were separated by periods of no activity (\square). The beginning and ending of each phase are defined by mean values (min.). Pseudocupulation continues beyond what is shown.

TABLE 1

Female behaviour during second phase of the courtship (A and B), and time elapsed until initiation of pseudocopulation (C) (cf. Fig. 1). P denotes the significance level between treatments in a Kruskal-Wallis one-way ANOVA.

Male treatment	A. Female raising her abdomen (min.)	B. Number of interruptions in the female's mating position	C. Pseudo- copulation initiated (min.)
	Mean (S.D.) N	Mean (S.D.) N	Mean (S.D.) N
Normal	11.2 (8.0) 18	1.6 (1.8) 11	39.0 (29.6) 18
Anaesthetized	9.2 (7.7) 8	2.4 (1.3) 8	23.3 (7.3) 8
Anaesthetized and leg amputated	12.4 (8.5) 20	1.9 (1.4) 20	32.6 (20.4) 20
Р	0.52	0.52	0.38

and lightly touched her legs. Almost half of the males (44%, N=18) started to bite off silk threads before they had touched the female. However, these males intensified web reduction activity after having touched the female. During the male's web reduction, the female raised her abdomen from a horizontal to a more or less perpendicular position (Tab. 1) (Fig. 1), producing an angle of approximately 60° between the ventral part of the abdomen and the horizontal plane. The female sometimes left this position (Tab. 1), e.g. after violent web reduction activity by the male. A quiet period followed the web reduction phase (Fig. 1). The male resided in the vicinity of the female and the pair often touched each other's forelegs.

During the third courtship phase, the pseudocopulation, normal copulation behaviour was performed but no sperm was transferred. The female, still with raised abdomen, waited in the reduced web. The male, waving his legs, approached the female whereupon the pair immediately assumed the mating position and pseudocopulation was initiated (Tab. 1) (Fig. 1). In some pairs, short breaks occurred at the beginning of the pseudocopulation phase. The pseudocopulation was initiated, on average, 1.8 ± 1.2 times (N=18) and the pair spent 1.6 ± 5.0 min. to find a stable mating position. However, more than half of the pairs (56%) succeeded in carrying out pseudocopulation the first time they entered the mating position (Fig. 2).

No female attacked or cannibalized her male partner during courtship or the rest of the cohabitation. All females that were fed after a period of 24 h together with the male oviposited and produced spiderlings.

Courtship by experimental males

All males that were treated by anaesthetization (N=8) and/or foreleg amputation (N=20) carried out the courtship. During the first and second phase of the courtship, we analysed the response of the female towards the male. We found no significant differences in the female's behaviour among the three groups of males, i.e. the time for the female's first mating position, number of interruptions, and the start of pseudocopulation (Tab. 1). During the third phase, however, short breaks at the beginning of the pseudocopulation were more common among males with an amputated foreleg. Only 25% (N=20) of these males managed to bring the pseudocopulation to an end in the first entered mating position (Fig. 2). When entering the mating position, they slipped down to the side and had difficulties to reach the female's epigyne. There were differences among the three groups in the number of mating positions initiated (P=0.056, Kurskal-Wallis one-way ANOVA, N=46) (Fig. 2). The groups with normal and anaesthetized males were similar (Multiple comparisons) (see Fig. 2), indicating that anaesthetization did not affect courtship behaviour. Therefore, they were pooled in further analyses. Males with amputated legs made more attempts (mean 4.4 ± 6.1 , N=20) before finding a stable mating position than pooled males (mean 1.8 ± 1.2) (P=0.018, Mann-Whitney U-test, N1=20, N2=26). Consequently, pairs in which the male had a shortened leg spent a significantly longer time (mean 10.9 ± 22.7 min. N=20) finding a stable mating position than pooled pairs (mean 1.2 ± 4.2 min. N=26) (P=0.022, Mann-Whitney U-test, N₁=20, N₂=26) (see also Fig. 1).



Number of Mating Positions Initiated



Male behaviour at the beginning of pseudocopulation. The number of mating positions initiated by males in treatment groups.

Number of Individuals

Males that had their right leg amputated (N=8) had more difficulties when entering the mating position than males with the left leg amputated (N=12). Males with a shortened right leg made more attempts (8.0 ± 8.6) before a pseudocopulation was completed than did males with a shortened left leg (2.0 ± 1.0) (P=0.027, Mann-Whitney U-test, N₁=8, N₂=12); They also spent more time (22.1 ± 31.6 min.) finding a stable mating position than did males with the left leg amputated (3.5 ± 10.3 min.) (P=0.018, Mann-Whitney U-test, N₁=8, N₂=12). Unfortunately, by chance the female's mean weight was lower within the group of males that had their right leg amputated (6.9 ± 0.9 mg) compared with the group of males that had their left leg amputated (8.2 ± 1.6 mg) (P=0.042, Mann-Whitney U-test, N₁=7, N₂=11). There were, however, no significant correlations between female weight and number of matings initiated, or time to find a stable mating position (P>0.5 and P>0.2, respectively, Spearman rank correlation test, N=42).

No female attacked her male partner during courtship. One anaesthetized male and two males with an amputated foreleg were eaten by the female within 24 h. All experimental females that were allowed to oviposit produced spiderlings, i.e. males were cannibalized after they had transferred sperm.

DISCUSSION

In *P. phrygianus*, females obviously allow injured males to mate although their courtship behaviour during pseudocopulation is different from that performed by normal males. Males with an amputated leg often seemed to have difficulties in finding appropriate position during the early phase of pseudocopulation. The female should have ample possibilities to reject an inferior male during courtship, and feeding on a male may even increase her future fitness. The female raised her abdomen before the male had finished the web reduction. It is not known what this behaviour means to the male. The female probably allows the male to start pseudocopulation but she has still the possibility to reject the male since no sperm has been transferred so far. Courtship among arthropods is usually performed in sequence and often the female has the option of rejecting the courting male at any stage (e.g. STEEL 1986*a*, *b*, ELGAR & NASH 1988). However, courtship may serve several functions and males and females often have conflicting interests even before the mating is initiated.

There may be several reasons why females do not reject injured males. First, there is a possibility that females rarely contact "damaged" males in nature and that they are unable to detect the injury. Second, there may not be any fitness gain in being discriminating. A third alternative is that the cost of rejection is too high.

How common are injured males in natural populations? If it is rare that males with injuries perform any courtship, then a discrimination by the females might be irrelevant. Out of 185 males collected for the present and other experiments, thirty specimens (16.2%) had one or more legs that were shortened or injured in some way. This suggests that males with leg injuries are common in the wild and that females may encounter such males quite often.

In the present study, we used males with strongly asymmetric forelegs. The forelegs are used in the communication between the sexes during courtship, i.e. the male touches the female lightly with the forelegs. The results indicated that asymmetric males performed "bad" courtship, acting clumsily during pseudocopulation. There are reasons to believe that females are able to detect the different behaviour of injured males in comparison with normal males. A study on *Linyphia litigosa* showed that males with injured or missing legs were avoided by remating females (WATSON 1993).

One reason to expect that females should reject males with an amputated leg is increased predation risks due to prolonged courtship. There were more attempts to start pseudocopulation in injured males than in normal ones. Consequently, injured males moved around in the web more frequently and the time until finding a stable mating positon was longer than for males without leg injuries. Thus, on average, an injured male performs a longer courtship which increases the exposure of himself and the female to visual predators. The pseudocopulation continues for a much longer time but then the male and female remain in mating position without moving around. The females may therefore be selected to reduce the courtship time. This was supported by the fact that females were willing to initiate the pseudocopulation (i.e. raising abdomen) before the males had finished web reduction. Presumably, the purpose of reducing the web area is to reduce the probability that male rivals will detect the female (data on other linyphiids, cf. ROVNER 1968, WATSON 1986), and consequently the courting male performs his task carefully.

The reason why females did not discriminate injured males is probably that the sex ratio is female biased in this species. *P. phrygianus* has a skewed primary sex ratio, averaging one-third of males and two-thirds of females (GUNNARSSON & ANDERSSON 1992). Furthermore, in severe winters, male survival declines considerably which may result in a sex ratio of 1 male: 9 females, or less (GUUNNARSSON 1987). This means that there is great annual variation in operational sex ratio, i.e. the ratio of sexually active females to males (EMLEN & ORING 1977) during the reproductive period in spring. Moreover, there are fluctuations in the operational sex ratio within the season as males mature before the females (GUNNARSSON & JOHNSSON 1990). A virgin female that is choosy may take a substantial risk to remain unmated. Under such a condition females should be selected to mate with the first male available.

It is unclear why males with their right foreleg shortened had more difficulties entering the mating position than had males with their left leg amputated. It suggests that one type of asymmetry is worse than the other. This may be a consequence of fine adjustment during the pseudocopulation due to signals between the sexes. Both palps are used during the pseudocopulation but it is unknown whether there are special sequences of left and right insertions. This warrants further investigation.

ACKNOWLEDGEMENTS

We thank C. Askenmo, J. Johnsson, J. Prenter, A. Schmitt, G. Uhl, P.J. Watson and one anonymous referee for comments on the manuscript. This work was supported by the Swedish Natural Science Research Council and the Foundations of A. Ahrenberg, and W. and M. Lundgren.

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